DISTRIBUTION AND EXCRETION OF ^{14}C-CYCLAMATE SODIUM IN ANIMALS

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Received August 29, 1966

Taylor et al. (1951) have studied the distribution and excretion of ^{35}S-labeled sodium cyclamate in animals while Schoenberger et al. (1953), have carried out similar studies in man. The latter human studies utilized both ^{35}S-labeled material as well as a gravimetric method reported by Audrieth and Sveda (1944). Both of these studies support the general concept of complete excretion of cyclamate salts, or possible metabolic products. ^{14}C-labeled sodium cyclamate has been synthesized in these laboratories and extensive studies have been, and are continuing to be, carried out concerning the distribution, excretion, and metabolism of sodium cyclamate. The ^{14}C-label was chosen to remove any doubt concerning the biological stability of the sulfur moiety of the cyclamate molecule. Using this material, we have demonstrated nearly quantitative and rapid excretion in both rats and dogs following either acute or semi-chronic administration. Further studies, using chromatographic methods, have shown that at least 98-99% of the excreted radioactivity, either fecal or urinary, is present as unchanged cyclamate.

Materials and Methods

The ^{14}C-labeled sodium cyclamate used in these studies was synthesized from uniformly ^{14}C-labeled aniline. Both chemical and radiochemical purity were carefully established using several chromatographic systems.

Biological assays were done on aliquots of feces or tissues, including skinned rat carcasses, which had been homogenized in a high-speed homogenizer. Blood and urine were sampled directly. Radioassay was accomplished using

either a modified Schöniger procedure described by Oliverio, Denham, and Davidson (1962) or a direct counting procedure described by Petroff et al. (1965). All studies are following oral administration of sodium cyclamate.

Thin-layer-chromatography studies were done using commercially-prepared plates which were plated with silica gel G. Plate scanning was accomplished using typical radioactivity detection equipment.

Results and Discussion

Excretion

1. Rats: Results of ^{14}C-cyclamate excretion studies in rats are summarized in the following table:

TABLE 1

^{14}C-Cyclamate Excretion Studies in Rats

No.	Dose (gm/kg)	Time (hrs)	Per cent of Administered Dose				
			Urine	Feces	Cage Wash	Total	Carcass
1	.909	24	22.66	67.61		90.27	
		48	2.73	2.94		5.67	
		72	0.34	0.03	1.93	2.30	0.08
						98.24	
2	.714	24	21.45	66.36		87.81	
		48	1.04	3.75		4.79	
		72	0.34	0.35	0.88	1.57	0.06
						94.17	
3	.833	24	17.41	76.6	2.66	96.67	0.41
4	6.67 (8 doses)	120				103.25	0.33
5	6.67 (8 doses)	120				102.59	0.24

2. Dogs: Acute studies in dogs administered either 200 or 1000 mg/kg showed urinary excretion of 43.4 and 27.2% of the administered dose within 4 days. At the same time, fecal excretion was 52.6 and 71.3% with total excretion values being 96.0 and 98.5% respectively.

A semi-chronic or equilibrium study was carried out in which an animal was administered 200 mg/kg of ^{14}C-cyclamate three times a day for a period

of 8 days at which time administration was ceased and excretion studies continued for 9 days. Approximately 40% of the dose was excreted in the urine, 54% in the feces, and excretion had essentially ceased within 2 days after stopping daily administration of the cyclamate.

The excretion data indicate rapid and complete elimination of the ^{14}C-labeled cyclamate following either acute or semi-chronic oral administration. Due to physical problems associated with excreta collection, it should be stressed that residual concentrations of a compound cannot be inferred from excreta data, but must come from direct assay of tissues per se, as summarized in the following section.

Distribution. Total carcass analysis in five rats 24-120 hours after doses of from 0.7-6.67 gm/kg showed maximum values of only 0.41% (see Table 1), which is consistent with the high recoveries in the excreta. Rats 1-3, which were administered comparable doses, demonstrate that total carcass levels drop from 0.41% at 24 hours to only about 0.07% of the dose at 72 hours.

Within 92 hours after a single dose of 200 mg/kg cyclamate, tissue concentrations in the dog were either not detectable or were extremely low. Significant radioactivity could not be detected in skin, eyes, testes, brain, thyroid, and three different muscle samples. Other organ concentrations, in per cent of dose per gram, were: spleen, 5.1×10^{-5}; liver, 3.6×10^{-5}; lungs, 17.3×10^{-5}; pancreas, 4.8×10^{-5}; heart, 3.6×10^{-5}; and kidneys, 5.7×10^{-5}.

A muscle biopsy sample (neck) was taken on day 8 from a dog which was administered 200 mg/kg three times per day for 8 days. Concentration in this sample was 3.8×10^{-4} per cent of the dose per gram. Nine days after ceasing administration an average concentration for muscle, from four sites, was only 2.7×10^{-5} %/gm. At this same time, organ concentrations were: lungs, 1.8×10^{-5}; liver, 1.2×10^{-5}; pancreas, 0.9×10^{-5}; and spleen, 1.2×10^{-5} per cent of the dose per gram.

Chromatography. We have investigated several thin-layer-chromatographic systems for the identification of Na cyclamate in excreta and find the following two systems the most satisfactory, i.e. 95% ethanol: 1N NH_4OH (95:5)-R_f 0.8 and acetonitrile: concentrated NH_4OH:water (60:10:3)-R_f 0.62.

Chromatographic studies on rat excreta indicate that ^{14}C-labeled cyclamate is excreted unchanged and that metabolism is negligible within detection limits of the plate scanner. Studies with the dog indicate that approximately 98% of the dose is excreted as unchanged cyclamate and that the remaining unidentified 2% may be a physical artifact caused by complexing or binding with extraneous urinary or fecal constituents. To support this latter hypothesis it should be mentioned that we can only detect this component in the acetonitrile system and that it is always associated with yellow urinary or fecal pigments. In these studies only about 40% of the dose is excreted in the urine so that the above 2% actually represents only about 0.8% of the administered dose. Attempts to characterize this component are actively continuing.

Volatility Studies. Many attempts have been made in our laboratories to detect the presence of possible volatile components within animal excreta. Such attempts have included freezing of excreta as collected, collection of excreta in acid solution, collection of respiratory CO_2 in basic traps, aeration of excreta with the air trapped in acid scrubbing towers, lyophilization, steam distillation, etc. All such attempts have indicated negligible amounts of volatile excretion products.

Biological Half-Life. Using available excretion data, we have calculated whole body retention and have estimated the biological half-life of orally-administered cyclamate from such data. Average half-life values are as follows: acute studies in 3 rats - average biological half-life 6.6 hrs., acute studies in 2 dogs - average biological half-life 8.8 hrs., and a semi-chronic study in 1 dog - average biological half-life 6.8 hrs. upon cessation of cyclamate administration. It should be noted that the biological half-

life of cyclamate is short enough that daily administration would not be expected to cause tissue accumulation. It should also be noted that prolonged semi-chronic administration to the dog did not appear to increase the biological half-life of the cyclamate.

Acknowledgements

We are indebted to Mr. Art Alter, radiochemist in these laboratories, for the synthetic preparation of the ^{14}C-labeled cyclamate and to Messrs. Carleton Pierce and Jerry Netwal for their valuable technical assistance.

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